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MINIREVIEW

53BP1, BRCA1, and the Choice between Recombination and End Joining at DNA Double-Strand Breaks

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When DNA double-strand breaks occur, the cell cycle stage has a major influence on the choice of the repair pathway employed. Specifically, nonhomologous end joining is the predominant mechanism used in the G_1 phase of the cell cycle, while homologous recombination becomes fully activated in S phase. Studies over the past 2 decades have revealed that the aberrant joining of replication-associated breaks leads to catastrophic genome rearrangements, revealing an important role of DNA break repair pathway choice in the preservation of genome integrity. 53BP1, first identified as a DNA damage checkpoint protein, and BRCA1, a well-known breast cancer tumor suppressor, are at the center of this choice. Research on how these proteins function at the DNA break site has advanced rapidly in the recent past. Here, we review what is known regarding how the repair pathway choice is made, including the mechanisms that govern the recruitment of each critical factor, and how the cell transitions from end joining in G_1 to homologous recombination in S/G_2.

DNA double-strand breaks (DSBs) are exceedingly dangerous chromosomal lesions. Failure to accurately repair DSBs can lead to gross chromosome rearrangements or mutations at the break site, which can cause cell death, cell transformation, and tumorigenesis. Two mechanistically distinct pathways have evolved to eliminate DSBs from the genome: nonhomologous DNA end joining (NHEJ) and homologous recombination (HR), both of which are conserved in all kingdoms of life. NHEJ entails the tethering of the broken DNA ends and their ligation (1). NHEJ is active throughout the cell cycle. While NHEJ accurately repairs "clean" DSBs whose ends are compatible and harbor undamaged terminal nucleotides, it is also capable of joining mismatched termini or termini that harbor damaged, otherwise-unligatable terminal nucleotides. In the latter case, joining is associated with DNA sequence loss. Moreover, when ends from two different chromosomes are joined, a chromosomal translocation ensues. In HR, the intact sister chromatid is most often engaged as the information donor. This process is normally accurate but requires that cells be in the S or G_2 phase of the cell cycle, when DNA replication generates the sister chromatid to direct the repair process.

How DSB repair pathway choice is determined at the molecular level has been the subject of intense study for quite some time. It has become clear that whether or not the DNA ends have undergone extensive 5′-to-3′ nucleolytic resection exerts a major impact on this choice, such that ends that bear long 3′ DNA tails become destined for HR repair (2, 3). As first revealed in genetic studies performed with the budding yeast *Saccharomyces cerevisiae*, three distinct nucleases function in the DSB end resection process: the 5′-to-3′ exonuclease I (Exo1), the flap endonuclease Dna2 in conjunction with the RecQ family helicase Sgs1 (BLM in humans), and Mre11, part of the Mre11-Rad50-Xrs2 (MRX) complex (MRE11-RAD50-NBS1, or MRN, in humans) (4, 5). MRX is required for the initiation of resection near the DSB, whereas Exo1 and Dna2 carry out long-range resection. There is evidence of cross talk between these pathways, as MRN and BLM both stimulate Exo1 *in vitro* (6, 7). The single-stranded DNA (ssDNA) binding protein RPA (replication protein A) coats the 3′ tails generated during resection, preventing the formation of secondary structures (8). RPA also stimulates Exo1 and directs DNA2 cleavage to the 5′ flap after unwinding by BLM (6, 9).

At DSBs with 5′ overhangs, resection eliminates the ability to align the ends for precise NHEJ (Fig. 1). DSBs with 3′ overhangs retain base-pairing potential after resection begins, but resection introduces extended gaps adjacent to the terminal nucleotides (Fig. 1). These DNA gaps have been shown to strongly inhibit NHEJ (10). A single-base gap reduces the NHEJ efficiency of otherwise-compatible overhangs severalfold, and accurate joining is prevented when the gap reaches 3 or 4 nucleotides (10). Thus, the onset of resection blocks restorative repair by NHEJ and instead favors DSB repair by HR. While accurate repair of the break by recombination is the preferred outcome, an error-prone backup pathway, referred to as the microhomology-mediated end joining (MMEJ) pathway, can also restore the DNA duplex (11). This pathway is mainly relevant when the ssDNA tails formed by resection fail to base pair fully, and microhomologies in the ssDNA tails instead form a short DNA hybrid to initiate the joining process (Fig. 1). MMEJ leads to deletion of the intervening sequence and is thus a highly mutagenic outcome.

The finding that resection can be inhibited by holding yeast cells in G_1 or inhibiting cyclin-dependent kinase (CDK) activity provided the first major clue into the mechanism by which pathway choice is regulated (12, 13). Later work identified Sae2 (CtIP in humans), an MRX/MRN interaction partner, as the critical CDK target responsible for this phenomenon (14, 15). A model has emerged in which CDK phosphorylates CtIP at the G_1-S transition to activate resection. In *Schizosaccharomyces pombe*, periodic expression of the CtIP homolog Ctp1 is also relevant for the cell cycle-dependent regulation of resection (16), and this mechanism seems to be conserved in mammalian cells (17). A second CDK target is the Dna2 nuclease, whose phosphorylation stimu-

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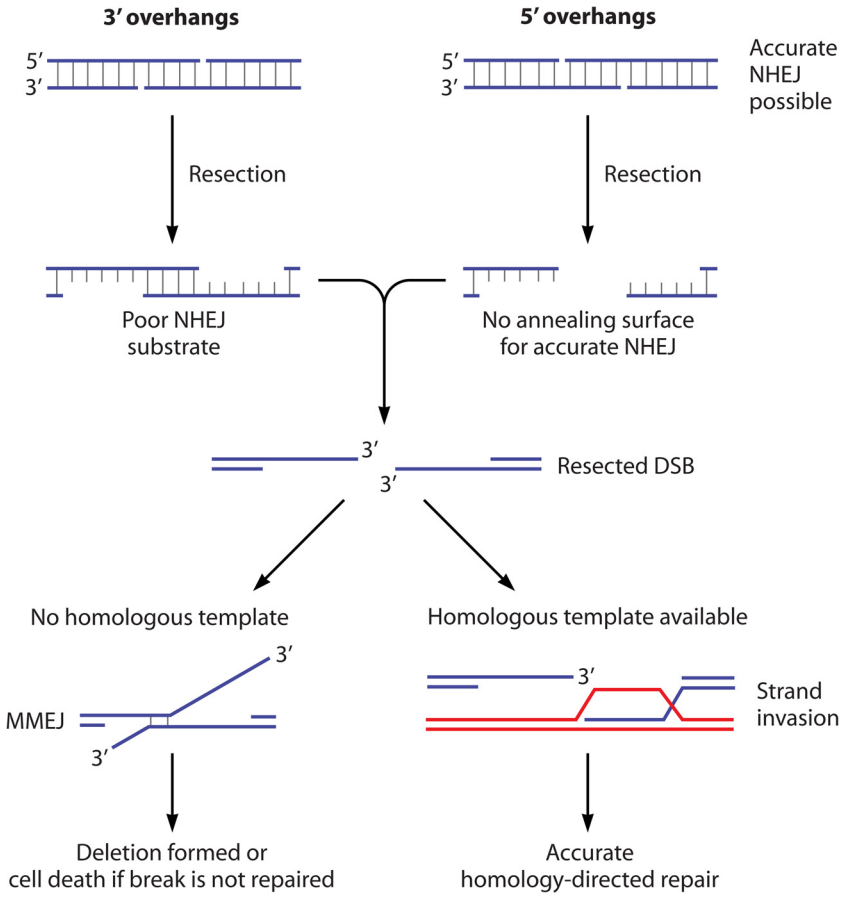

FIG 1 The effects of nucleolytic resection on NHEJ of DNA ends that bear 5′ or 3′ overhangs. With ends that harbor 3′ overhangs (top left), NHEJ becomes inefficient once the gap size stemming from resection becomes 2 nucleotides or greater. Accurate NHEJ of ends with 5′ overhangs (top right) is eliminated by resection. After resection, repair normally proceeds by HR (bottom right). Occasionally, and especially when a homologous template is unavailable, deletion-prone MMEJ occurs (bottom left).

lates its recruitment to DSBs (18). The above findings suggested a straightforward model in which a phospho-dependent switch at the G_1-S transition turns on resection. However, further investigations have revealed much greater complexity in the determination of DSB repair pathway choice, which involves DNA damage checkpoint proteins, the breast cancer susceptibility gene BRCA1, telomeric factors, ubiquitylation and SUMOylation cascades mediated by RING finger proteins, and a variety of histone modifications. While the basic mechanisms of HR and NHEJ are conserved, many of the mammalian factors that have been characterized in pathway choice do not have a clear ortholog in *S. cerevisiae*, suggesting that this aspect of the DNA damage response has diverged considerably in higher organisms. Indeed, blocking of resection in the G_1 cell cycle phase seems to be more robust in mammalian cells than in yeast, as residual resection can occur during G_1 arrest in yeast (19, 20). Here, we review these findings and strive to integrate them in a mechanistic model.

REGULATION OF THE ONSET OF RESECTION BY 53BP1 AND BRCA1

A series of studies published in 2010 implicated 53BP1, a target of the ATM kinase that forms nuclear foci upon DSB induction, and the tumor suppressor BRCA1 in DNA end resection control. 53BP1 was shown to negatively regulate resection in G_1 (21). Importantly, BRCA1 promotes the removal of 53BP1 in S phase to allow resection (22). Consequently, in cells lacking BRCA1, resection is not upregulated in S phase and inappropriate NHEJ occurs at replication-associated DSBs, leading to gross chromosomal rearrangements. In mice, deletion of 53BP1 suppresses the embryonic lethality and prevents the chromosomal rearrangements seen in BRCA1$^{-/-}$ animals, emphasizing the importance of BRCA1-dependent removal of 53BP1 to facilitate the transition from NHEJ to HR (22–25).

The above findings raise new questions. Numerous proteins have been found that colocalize with γ-H2AX foci, which mark DNA damage sites. Some of these factors function upstream of both 53BP1 and BRCA1 recruitment, and how they promote or suppress either of the two repair pathways is the subject of ongoing investigations. What role do chromatin modifications play in recruitment of key factors? What is the molecular switch that activates BRCA1's ability to remove 53BP1 exclusively in S/G_2? Moreover, what is the mechanism by which 53BP1 blocks the resection nucleases? Below, we analyze recent findings that help address these questions.

UPSTREAM ACTION OF RNF8 AND RNF168 UBIQUITIN LIGASES

ATM, MDC1, the MRN complex, and the RING finger E3 ubiquitin ligases RNF8 and RNF168 are among the earliest factors

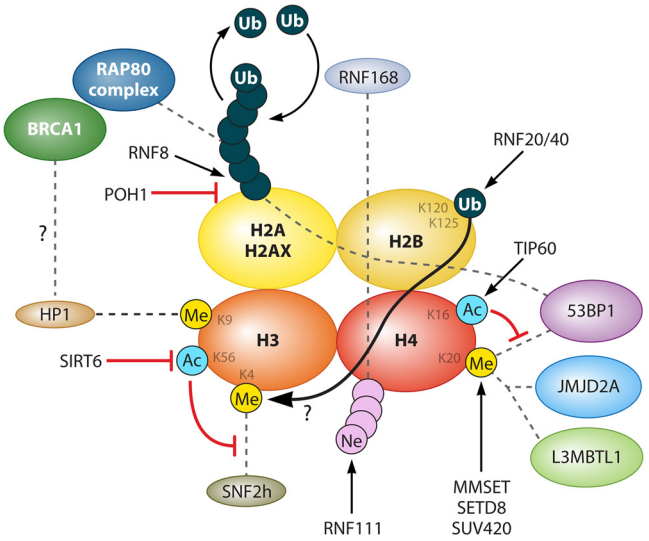

FIG 2 Histone modifications involved in 53BP1 or BRCA1 recruitment to DSBs. Proteins that add or remove each modification are indicated by arrows. Dotted lines indicate protein-protein interactions. A question mark indicates a relationship that has been documented in the literature but whose mechanism is unknown.

found in DNA damage foci (26). 53BP1 and BRCA1 appear later, and their recruitment is dependent on the aforementioned upstream factors (27). RNF8 is recruited via an interaction between its FHA domain and phosphosites on MDC1 (27, 28). Acting with its partner E2 enzyme UBC13 (29), RNF8 ubiquitinates γ-H2AX (28) and H2A (27). This, along with other chromatin modifications that occur near the DSBs, is summarized in Fig. 2. RNF168 is then recruited, which also partners with UBC13 and further propagates the ubiquitination of histone γ-H2AX and of lysines 13 and 15 of histone H2A (30, 31). It should be noted that RNF8 and RNF168 form K63-linked ubiquitin chains which, unlike K48-linked chains, do not lead to protein degradation by the proteasome. Instead, the ubiquitinated targets act as recruitment platforms for downstream factors, which will be discussed in detail below. RNF168 recruitment is partially dependent on RNF111 and UBE2M, which are E3 and E2 enzymes, respectively, that conjugate the ubiquitin-like protein NEDD8 to a cluster of lysine residues on the N terminus of histone H4 (32). RNF168 physically interacts with "polyneddylated" H4, which is thought to be crucial for its DSB recruitment (32).

Two deubiquitinating enzymes (DUBs), USP34 and OTUB1, regulate the level of RNF168 and the activity of the UBC13-RNF168 complex, respectively. The level of RNF168 in cells is controlled by its ubiquitin-dependent proteolysis. USP34 enhances the abundance of RNF168 by deubiquitinating it (33). Accordingly, cells deficient in USP34 are unable to mount DNA damage-induced 53BP1 and BRCA1 foci (33). In this regard, USP34 acts as a positive regulator of RNF168. OTUB1, on the other hand, negatively regulates RNF168 activity, although the mechanism is, surprisingly, independent of its DUB activity (34, 35). OTUB1 physically interacts with and attenuates the ubiquitin-conjugating activity of UBC13, the E2 partner for both RNF8 and RNF168. While OTUB1 restricts the activity of both UBC13-RNF8 and UBC13-RNF168 *in vitro*, only RNF168-dependent events seem to be affected *in vivo*. The end result is that OTUB1 negatively regulates HR. Its depletion alleviates the HR defect seen in cells treated with an ATM inhibitor, and OTUB1 overexpression inhibits HR (35). Finally, RNF168 activity is kept in check by the E3 ubiquitin ligases TRIP12 and UBR5, which prevent histone ubiquitination from spreading beyond the region surrounding the DSB (36).

Recently, RNF168 has also been shown to interact with the A and B isoforms of the E2 ubiquitin-conjugating enzyme RAD6 (37). These are orthologs of yeast Rad6, which helps mediate postreplication DNA repair, proteolysis via the N-end rule pathway, and other processes (38). Both RAD6 isoforms are required for 53BP1 and BRCA1 recruitment to DSBs (37). Liu et al. (37) implicated the RAD6A/B-RNF168 complexes in ubiquitination of the linker histone H1.2. However, the biological relevance of H1.2 ubiquitination remains to be established. It seems likely that other proteins are targeted by ubiquitination during the DNA damage response, and further work will be needed to identify and characterize the contribution of novel targets.

Besides ubiquitination, SUMOylation also plays a role in the RNF8-RNF168 pathway. SUMO, UBC9 (the SUMO E2-conjugating enzyme), and the SUMO ligases PIAS1 and PIAS4 all localize to DNA damage, and the recruitment of both 53BP1 and BRCA1 requires PIAS1 and PIAS4 (39, 40). While RNF8 localization occurs efficiently in the absence of either PIAS protein, PIAS4 is required for RNF168 localization and establishment of H2A ubiquitin adducts (39). While these results identify the step in the cascade where PIAS4 acts, it is unclear where PIAS1 functions. How PIAS1 and PIAS4 are recruited to DNA damage is also unknown, beyond the requirement of MDC1 for their localization (39). BRCA1 and 53BP1 have both been identified as likely PIAS1/PIAS4 targets, but how SUMOylation affects the properties of these factors is not yet known (39, 40). Given that SUMOylation affects H2A ubiquitination, an event upstream of 53BP1 and BRCA1 recruitment, there are likely other SUMOylation targets that remain to be identified.

THE RAP80 COMPLEX AND RNF20/RNF40-DEPENDENT RECRUITMENT OF BRCA1

Ubiquitination of H2A by RNF8 and RNF168 is thought to provide a recruitment platform for RAP80 and its associated proteins, BRCC36, Abraxas, MERIT40, and BRCC45 (27, 41–46). RAP80 directly interacts with ubiquitin and presumably with ubiquitinated H2A, thereby nucleating the assembly of a higher-order complex that harbors the aforementioned partner proteins (Fig. 2) (42, 44, 45). All members of the RAP80 complex are required for each other's stability and for the optimal recruitment of BRCA1 to DSBs, which is contingent upon its direct interaction with Abraxas (41, 43, 45). BRCC36 is a DUB that specifically targets K63-linked ubiquitin chains (47). The ubiquitin content of γ-H2AX foci peaks early and then it decreases in a manner that is dependent on the RAP80 protein complex, suggesting that the latter counteracts the actions of RNF8 and RNF168 (47). While loss of RAP80 reduces BRCA1 foci, it, paradoxically, increases long-range resection as well (48). A possible explanation is that deubiquitination of H2A affects the resection nucleases differently, e.g., stimulating MRN-dependent resection near the DSB but negatively regulating long-range resection by BLM-DNA2 and/or EXO1. This is an important topic that should be addressed.

It has been suggested that removal of K63-linked Ub chains from H2A by the RAP80 complex is part of an intricate process

that helps convert them to K6-linked chains catalyzed by the E3-ubiquitin ligase activity of the BRCA1-BARD1 complex (41). Consistent with this idea, RAP80 has affinity for K63- and K6-linked but not K48-linked ubiquitin chains (44). However, a recent report showed that a BRCA1 RING domain mutant that was defective in E3 ligase activity supported resection at the wild-type level (49). This study revealed that, if BRCA1 does indeed form K6-linked chains at DSB sites, they are dispensable for efficient resection.

The RING finger E3 ubiquitin ligases RNF20 and RNF40 also function upstream of BRCA1 recruitment, but the mechanism is less clear. These ligases modify histone H2B on lysines 120 and 125 (Fig. 2) (50). RNF20 knockdown causes a delay in the recruitment of the general recombinase RAD51 and its partner proteins to DNA damage, and an H2B point mutant that cannot be ubiquitinated exerts a stronger effect in this regard (50, 51). NBS1, an integral component of the MRN complex, interacts directly with RNF20, and this interaction is required for resection (51). However, H2B ubiquitination occurs efficiently in the absence of MRN, arguing against an involvement of MRN in RNF20-mediated H2B modification (51). RNF20 is also required for methylation of lysine 4 in histone H3 (51). This methylation mark, alongside the deacetylation of lysine 56 in H3 by SIRT6, leads to recruitment of the SNF2h nucleosome remodeler, which is required for optimal resection efficiency (51, 52). Intriguingly, the RNF20 resection defect can be overcome by treating cells with chloroquine, which leads to chromatin relaxation (51). Thus, RNF20 and RNF40 may promote histone eviction, an important step, considering that nucleosomes pose an obstacle to resection nucleases, as revealed in biochemical reconstitution studies (53). Indeed, chromatin immunoprecipitation experiments have yielded results suggesting that histones are removed near the DSB site within a time frame consistent with resection (54, 55). BRCA1 recruitment is also reduced in the absence of HP1, which interacts with H3 methylated on K9 (56). Further work is needed to determine the mechanism by which HP1 influences BRCA1 activity.

RECRUITMENT OF 53BP1

How 53BP1 is recruited to DSB sites has been debated. While it has been reported that 53BP1 recruitment requires its interaction with the tandem BRCT domains of MDC1 (57, 58), other studies have found the proposed MDC1 interaction domain to be dispensable in this regard (59, 60). 53BP1 also interacts with histone H4 dimethylated on lysine 20 (H4K20me2) via its Tudor domain (Fig. 2), but early studies did not detect an increase in H4K20me2 upon DNA damage (61). This suggested a model in which 53BP1 is initially recruited by MDC1 and is then retained in the chromatin region surrounding the break through an interaction with H4K20me2, which is presumed to be exposed following damage (61). This model is consistent with recent reports implicating PR-SET7/SETD8 and SUV420 in 53BP1 recruitment (62, 63). An alternate model has been proposed in which the methyltransferase MMSET promotes a localized increase in H4K20me2 near the DSB site (64). Like 53BP1, MMSET is phosphorylated by ATM upon the occurrence of DNA damage and appears to be recruited via an interaction with the BRCT domains of MDC1 (64). Further work will be needed to resolve conflicting data in this area. 53BP1 also harbors a C-terminal ubiquitin-interacting motif that recognizes histone H2A ubiquitinated on K15, a modification that is catalyzed by RNF168 (Fig. 2) (59). This finding helped explain why RNF8 and RNF168 are required for 53BP1 recruitment, but the RAP80 complex, which functions immediately downstream, is not (47). Furthermore, acetylation of histone H4 on lysine 16 by TIP60 has been shown to reduce the affinity of 53BP1 for H4K20me2 (Fig. 2) (65). Thus, three separate histone modifications—methylation, ubiquitination, and acetylation—are involved in regulating the recruitment and retention of 53BP1 (59).

Additional layers of regulation are afforded by two proteins, JMJD2A and L3MBTL1, which compete with 53BP1 for binding to H4K20me2 (Fig. 2) (66–68). JMJD2A interacts with H4K20me2 via its tandem Tudor domains, whereas L3MBTL1 utilizes an MBT domain to accomplish this feat. The RNF8-RNF168 pathway also has a role in the removal of JMJD2A and L3MBTL1 from DSB regions. Specifically, RNF8 and RNF168 directly ubiquitinate JMJD2A to trigger its degradation (68), and RNF168 also ubiquitinates VCP, which has ATPase activity, causing it to relocalize to chromatin, where it removes L3MBTL1 (66). The proteasome-associated DUB POH1 antagonizes RNF8-RNF168 activity on JMJD2A, promoting the stability of the latter and attenuating 53BP1 recruitment (67). POH1 likely targets other proteins too, as it has been implicated in RAD51 accumulation, a process that is independent of (and is, in fact, antagonized by) 53BP1 (67). Advanced microscopy has shown that, as cells transition into S/G_2, POH1 promotes the removal of RAP80 from DSB sites (49). As discussed above, the recruitment of RAP80 is dependent on RNF8/RNF168-mediated ubiquitination of histone H2A. Interestingly, the DUB activity of BRCC36, an integral component of the RAP80 complex, counteracts RNF168 to help achieve an equilibrium state of ubiquitination. An attractive model is that POH1 removes the initial ubiquitin conjugated to H2A, terminating the chain extension/reduction cycle maintained by RNF168 and RAP80 (Fig. 2).

The BLM helicase has recently also been implicated in 53BP1 recruitment. Cells from Bloom syndrome patients fail to recruit 53BP1 to DNA damage sites (69). BLM-deficient cells also show evidence of increased resection during microhomology-mediated end joining, which is surprising given that BLM helps catalyze one branch of long-range resection (69). These results suggest a dual role for BLM: an early antiresection function with 53BP1 and a late proresection role with DNA2 after 53BP1 has been removed. Separation-of-function BLM mutants will be critical in further characterizing this dichotomy.

RIF1 AND PTIP, KEY EFFECTORS OF 53BP1

The N terminus of 53BP1 is phosphorylated by ATM after DNA damage, but the phosphorylation sites are not required for 53BP1 localization to nuclear foci. Instead, these sites are necessary for resection attenuation, suggesting that phosphorylation of 53BP1 is important for the recruitment of effector molecules (70). A series of papers published in 2013 identified RIF1 as a 53BP1 effector (Fig. 3A) (71–74). Like 53BP1, RIF1 is required for NHEJ and is removed from foci in S/G_2 in a BRCA1- and CtIP-dependent manner. 53BP1 and RIF1 coimmunoprecipitate from cell extracts (73), but a direct interaction *in vitro* with purified proteins has not yet been reported, so it remains possible that an adapter links these two proteins together.

The observation that loss of RIF1 only partially restores HR in cells lacking BRCA1 (75) suggested that another protein may be involved in the attenuation of HR to favor NHEJ. Indeed, PTIP

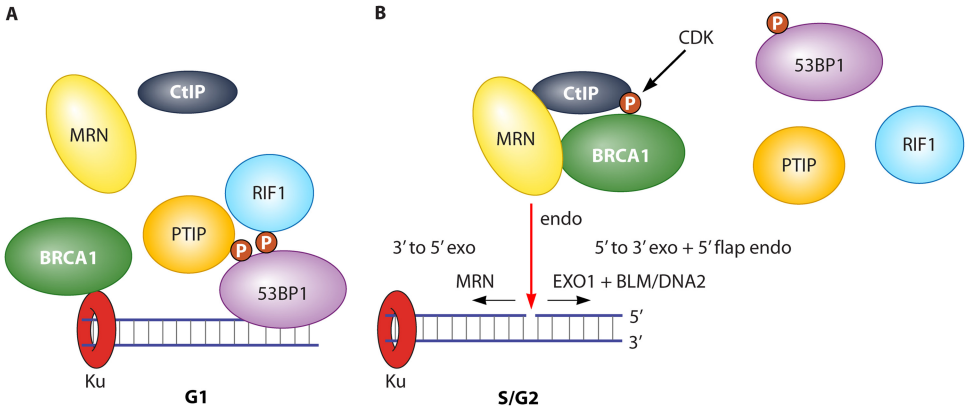

FIG 3 Model for DSB occupancy in G_1 and S/G_2. (A) In G_1 cells, 53BP1 is phosphorylated by ATM and becomes localized at the break. RIF1 and PTIP are recruited in a phospho-dependent manner and block resection via an unknown mechanism. BRCA1 is bound to Ku but in a quantity so low that they do not appear as visual foci. (B) In S/G_2, CtIP is phosphorylated by CDK, inducing the formation of a complex with BRCA1 and MRN. This complex displaces 53BP1 and initiates resection. Ku may be removed by coordinated endo- and exonuclease activities that are initiated from a nick in a manner analogous to the postulated mechanism of Spo11 removal.

has recently been identified as an additional 53BP1 effector (76). Using its tandem BRCT domains, PTIP interacts directly with 53BP1 in a manner that is dependent on the ATM-mediated phosphorylation of serine 25 of the latter (76). Like RIF1, loss of PTIP restores RPA foci in BRCA1-deficient cells, indicating that PTIP also plays a role in blocking resection (76). PTIP has also been reported to bind γ-H2AX, but the functional significance of this is not yet clear (77).

The mechanisms by which RIF1 and PTIP prevent resection and promote NHEJ have not yet been elucidated. However, it is known that RIF1 interacts with the BLM helicase and is required for recruitment of BLM to foci (75, 78). Conceivably, RIF1 may inhibit the ability of BLM to unwind DNA for resection by DNA2. Or, RIF1 and/or PTIP may stabilize NHEJ proteins, such as the Ku heterodimer on DNA ends, which would prevent access of the ends for the resection nucleases (see below). The DNA end structure may help determine the activities of RIF1 and PTIP. A key difference between the two effectors is that PTIP seems specific to the inappropriate S-phase NHEJ events that characterize cells lacking BRCA1, while RIF1 is additionally required for class switch recombination, a specialized form of NHEJ that occurs at DSBs generated by cytosine deamination in developing B cells. Interestingly, V(D)J recombination, a form of NHEJ that joins hairpin-capped ends during lymphocyte development, is unaffected by the loss of RIF1 (71) but is defective in cells lacking PTIP (79). Also, NHEJ of blunt-ended leading-strand telomeres is more dependent on 53BP1 than lagging-strand telomeres, which have 3′ overhangs (80). Uncovering the mechanisms of resection inhibition by RIF1 and PTIP will be a top priority in the near future.

THE G_1-S TRANSITION: CtIP PHOSPHORYLATION AND REMOVAL OF 53BP1 BY BRCA1

The mechanism by which BRCA1 helps mediate 53BP1 removal as cells transition from G_1 into S phase is unclear. However, it is important to note that the loss of BRCA1 leads to the DSB recruitment of 53BP1 in G_2 (73), suggesting that the 53BP1 recruitment platform remains intact in S/G_2 but is somehow masked by BRCA1. Similarly, 53BP1 knockdown leads to ectopic BRCA1 nuclear foci in G_1 (73), indicating that the potential for BRCA1 recruitment exists in G_1 but is blocked in a 53BP1-dependent manner.

How, then, do cells switch from pro-NHEJ 53BP1 in G_1 to proresection BRCA1 in S/G_2? Existing evidence hints at CtIP as the key mediator of this switch. CtIP phosphorylation by CDK is required for the initiation of resection in S phase (81). A G_2-specific complex containing CtIP, MRN, and BRCA1 has been reported (82, 83). CDK activity is required for the assembly of this higher-order complex (82). In a unified model, formation of the CtIP-MRN-BRCA1 complex triggers 53BP1-RIF1 removal and resection initiation (Fig. 3B). The finding that RIF1 could not be removed from foci in a CtIP phosphorylation site mutant is certainly consistent with this idea (73). Depletion of 53BP1 or RIF1 restores resection in BRCA1-deficient cells but not in cells lacking CtIP (34). These results reveal that CtIP has other functions beyond 53BP1-RIF1 removal (81) and that removal of 53BP1-RIF1 is the critical function of BRCA1 in resection. The observation that phosphorylated CtIP is ubiquitinated by BRCA1 led to the suggestion that this CtIP modification promotes resection (84). However, the recent finding that a BRCA1 RING domain mutant is still competent for resection casts doubt on models in which ubiquitination of downstream targets by BRCA1 is important for 53BP1 removal (49). Instead, 53BP1 displacement seems to be a separate activity of BRCA1, with the E3 ubiquitin ligase activity provided by RNF8 and RNF168 being most relevant to resection.

The observation that BRCA1 foci are impaired but not abolished in the absence of the RAP80 complex indicates that there is another means of BRCA1 recruitment independent of the BRCA1-Abraxas interaction (41, 43, 48, 85). Specifically, the FHA and BRCT domains of NBS1 interact with MDC1 that has been phosphorylated by casein kinase 2, and this interaction could be sufficient to tether the BRCA1-CtIP-MRN ensemble to the damage site when the RAP80 complex is missing (86).

Evidence from yeast has identified the chromatin remodeler Fun30 (SMARCAD1 in humans) as another possible player in 53BP1 removal (87–89). Fun30 is especially important for resection when Rad9, the yeast ortholog of 53BP1, is present (87). It

will be important to investigate genetic interactions between SMARCAD1 and members of the 53BP1-BRCA1 pathway.

REMOVAL OF Ku FROM DSB ENDS

Ku is a conserved, toroidal heterodimeric molecule consisting of two subunits, Ku70 and Ku80. It is abundant in the nucleus and has high affinity for DNA ends. The engagement of DNA ends by Ku represents the key first step in NHEJ (90). Genetic experiments in yeast have shown that Ku occludes DNA ends from Exo1. Ku and MRX/MRN are among the first proteins recruited to DSBs (91, 92). Importantly, the MRX nuclease activity and Sae2 (orthologous to CtIP) function in conjunction to remove Ku (92–95). Insights into how the MRX nuclease might release Ku from DNA ends have emanated from studies on meiotic recombination initiation. Early on in the meiotic program, the topoisomerase-like protein Spo11 acts in a genome-wide fashion to introduce DSBs into chromosomes to trigger HR. This serves to tie homologs together to prepare them for segregation in the first meiotic division. After mediating DNA strand breakage, Spo11 remains covalently bound to the DSB ends. A plausible model for Spo11 removal involves endonucleolytic incision of the DNA adjacent to the end by the MRX complex, followed by bidirectional exonucleolytic digestion from the nick, with MRX acting 3′ to 5′ (toward the break end) and Exo1 or Dna2/BLM performing long-range 5′-to-3′ resection (96). The use of small-molecule inhibitors of MRN endo- and exonuclease activities has provided support for a similar model at mitotic DSBs, where Spo11 is absent but Ku is likely to be bound (97). Whether or not the human MRN complex can overcome resection inhibition by Ku in a manner analogous to Spo11 is being addressed by different groups (98, 99). It is also unclear whether Ku exerts as much of an inhibitory effect on resection in mammalian cells as it does in yeast (24).

The identification of an interaction between BRCA1 and Ku provided support for a mechanism by which MRN, in complex with BRCA1 and phospho-CtIP, mediates Ku removal (Fig. 3B) (100). More recent results have shown that the BRCA1-Ku interaction is specific to the G_1 phase, however, and is important for NHEJ fidelity (101). These results reveal that BRCA1 is in fact present at the DSB site during G_1, even though it does not form microscopically visible foci until S/G_2. It will be critically important to determine whether the Ku-interacting form of BRCA1 is part of the aforementioned complex with MRN and CtIP or whether separate complexes that fulfill different functions exist. It is possible that BRCA1 associates only with Ku in G_1 but forms a distinct complex with MRN and CtIP upon entry into S phase in order to activate the MRN nuclease activity to initiate resection. The N terminus of BRCA1 interacts with both Ku and Nbs1, but mutants differentially affected for either interaction are not yet available (82, 100, 101). Whether the MRN nuclease activity is required for Ku removal in mammalian cells remains an open question, but recent advances in assessing Ku localization should allow this important experiment to be done (102).

Another possibility is that BRCA1, in a manner analogous to 53BP1, functions through effector molecules to promote resection. One candidate for such a factor is the telomere protein TRF1, which has recently been shown to localize to foci downstream of BRCA1 to promote resection (103). Alleles that separate the telomeric function of TRF1 from this resection role will be beneficial for the further characterization of this factor in resection.

CONCLUDING REMARKS

In *Escherichia coli*, the RecBCD complex, which harbors both helicase and nuclease activities, is the major protein machine that mediates resection. An early assumption was that eukaryotes employ a similar compact, efficient resection enzyme ensemble. The failure of early radiation sensitivity screens in yeast to identify "the" resection nuclease was a clue that resection is a great deal more complicated in eukaryotes. It is fair to say that no one anticipated the level of complexity that has been uncovered to date, though. Besides the involvement of three distinct nucleases, layers upon layers of intricate regulation have been identified in regard to making the correct choice between NHEJ and HR.

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